

changes in order may not be characteristic of anti-R and anti-R' serum, since different pools of the same antiserum are observed to give somewhat different results.

The importance of the phenyl group is shown by the fact that methylarsonic acid has practically no inhibitory power.¹⁰ On the other hand, the haptens $\text{HOC}_6\text{H}_4\text{NNC}_6\text{H}_4\text{AsO}_3\text{H}_2$ and $\text{H}_2\text{NC}_6\text{H}_4\text{NNC}_6\text{H}_4\text{AsO}_3\text{H}_2$, which are very like the immunizing haptenic group $-\text{NNC}_6\text{H}_4\text{NNC}_6\text{H}_4\text{AsO}_3\text{H}_2$, show very strong inhibition.

The very strong inhibition shown by the trihaptenic substance VI is probably due to the entropy effect of the three haptenic groups.

The fact that this substance and the other polyhaptenic substances containing R groups give at most only slight precipitates with anti-R' serum we attribute to steric repulsion of antibodies; their strong inhibitory action shows that a strong bond can be formed with one antibody molecule. Presumably a group R with part of the nucleus to which it is attached occupies the cavity of an anti-R' antibody; the remaining groups R then protrude from the antibody by such a small distance that another antibody cannot approach closely enough to form a bond. This steric effect is similar to that invoked earlier³ in explanation of the observed effective bivalence of trihaptenic

(10) See also F. Hauowitz and F. Breinl, *Z. physiol. Chem.*, **214**, 111 (1933).

and tetrahaptenic antigens. The formation of an appreciable amount of precipitate by the exceptional substance IX we attribute to the large size of its molecules, in which arsonic acid groups are separated by six benzene rings. The same explanation—large distances between haptenic groups—applies to the precipitation of anti-R' serum by R-ovalbumin (Table II).

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Summary

Quantitative data are reported for the precipitation reactions of polyhaptenic simple substances and antisera prepared by inoculating rabbits with azoproteins made from *p*-(*p*-azophenylazo)phenylarsonic acid. Measurements were made of the inhibitory effect of each of twenty-six haptens on one antigen-antibody reaction, and interpreted to give values of the bond-strength constant of the haptens with the antibody. These values are discussed in relation to the structure of the hapten molecules and in comparison with the values previously found for an antiserum homologous to the *p*-azophenylarsonic acid haptenic group. An explanation in terms of steric hindrance is given of the failure of some polyhaptenic substances to give precipitates with the antiserum.

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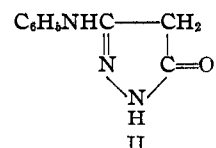
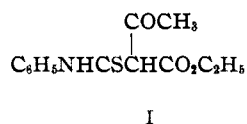
[COMMUNICATION NO. 906 FROM THE KODAK RESEARCH LABORATORIES]

Investigation of Pyrazole Compounds. III.¹ The Condensation of α -Carbethoxyacetothioacetanilide with Hydrazines

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By the condensation of phenyl isothiocyanate with sodium acetoacetic ester suspended in ether, Worrall obtained the sodium salt of α -carbethoxyacetothioacetanilide I.² The free ester on heating with two moles of hydrazine formed a compound which may be formulated as 3-anilino-5-pyrazolone II.³ The over-all reaction is represented in III (R = H).

In the present work we have modified Worrall's procedure in that instead of isolating the free ester I, we added one molecular proportion of

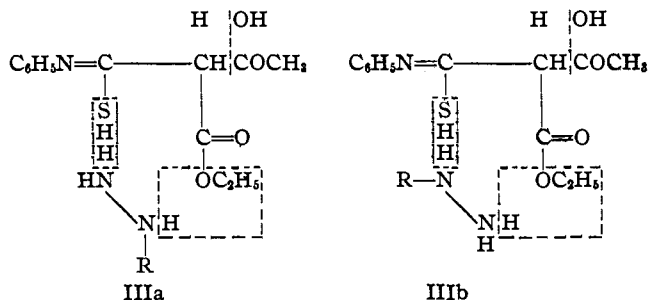


hydrazine to the alcoholic reaction mixture containing the sodium salt of I. A compound was obtained of the composition of 3-anilino-5-pyrazolone, with a melting point 268–270° (dec.). The same material resulted when Worrall's procedure was followed strictly, but this author reports a melting point of 255–256° (dec.). The difference does not appear to be caused by the technique of measurement and may indicate polymorphism.¹

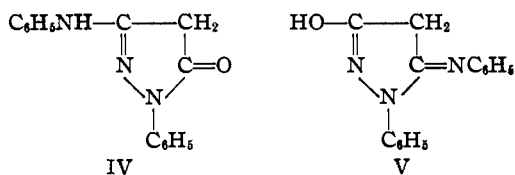
(1) Investigation of Pyrazole Compounds, II, *THIS JOURNAL*, **66**, 52 (1943).

(2) Worrall, *ibid.*, **40**, 418 (1918).

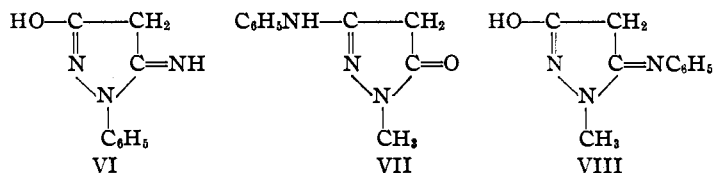
(3) Worrall, *ibid.*, **44**, 1551 (1922).



It can be seen from IIIa and b that an alkyl or aryl hydrazine, when condensed with I, may give two isomers: *e. g.*, phenylhydrazine may form the 1-phenyl-3-anilino-5-pyrazolone IV or the 1-phenyl-3-hydroxy-5-pyrazolone anil V. Worrall was not successful in isolating any pyrazolone from the reaction of I with phenylhydrazine, the only product obtained being β -acetylphenylhydrazine. With the change in the procedure mentioned above and a prolonged reaction time, we obtained, in this condensation, a compound melting at 168° which analyzed correctly for IV or V. Since it is different from the previously described compound IV⁴ (m. p. 218°), the new compound was considered to be V. The same compound was obtained by heating 1-phenyl-3-hydroxy-5-pyrazolone imide VI with aniline, a synthesis which confirms structure V.

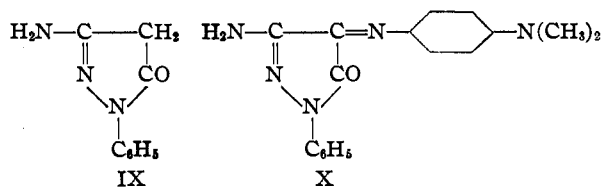


When methylhydrazine was condensed with the sodium salt of I, following the new procedure, two compounds resulted with m. p. 208° and 220°, respectively. Their identical analysis suggested that one is 1-methyl-3-anilino-5-pyrazolone, VII,



and the other, 1-methyl-3-hydroxy-5-pyrazolone anil VIII. Both compounds, like the compounds IV and V, and the parent substances of the latter, 1-phenyl-3-amino-5-pyrazolone IX, and VI, form dyes when oxidized in the presence of *p*-aminodimethylaniline.¹ However, VI, V, and the com-

pound of m. p. 208° yield only a weak, dull, bluish-magenta, in contrast to the brilliant magenta abundantly formed by IX, IV, and the compound of m. p. 220°. A division into the same two categories was made by the reaction of these compounds with *p*-nitrosodimethylaniline in boiling ethanol. In the beginning, the solution had the green color of the nitroso compound, but those containing IX, IV, and the compound with the m. p. 220°, rapidly turned a deep red, as should be expected when a magenta dye was formed, while no dye formation was observed with VI, V, and the compound with the m. p. 208°. It is, therefore, concluded that the compound with m. p. 220° is VII, while the compound of m. p. 208° is VIII. The dyes are obviously azomethine dyes, as exemplified by X.



Acknowledgment.—We are indebted to Mr. E. C. Armstrong, who worked out the color tests.

Experimental

3-Anilino-5-pyrazolone,³ I.—1. To a solution of sodium methylate (8.8 g. of sodium in 120 ml. of methanol) was added 50 g. of ethyl acetoacetate and then 52 g. of phenyl isothiocyanate in a thin stream at room temperature. The mixture was refluxed for half an hour, cooled to about 50° and 24 g. of 85% hydrazine hydrate added in a thin stream while stirring. After refluxing gently for one hour, the mixture was cooled, filtered and the product washed with 50 ml. of 95% ethanol to give 51 g. (76.5%) of white flakes; m. p. 268–270° dec. when heating at a rate of 5°/minute. Taken at the conventional rate of 2°/minute, the m. p. is merely spread, 262–268° dec. A small sample recrystallized from a large volume of ethanol had the same melting point.

Anal. Calcd. for $C_8H_8N_3O$: N, 24.0. Found: N, 23.82.

2. The two-step procedure of the literature² gave an over-all yield of 51% of 3-anilino-5-pyrazolone,³ m. p. 268–270° dec.

3-Anilino-5-pyrazolone is soluble in 3% sodium carbonate and sparingly soluble in 2% hydrochloric acid.

1-Phenyl-3-hydroxy-5-pyrazolone Anil, V.—1. To a solution of sodium methylate (5 g. of sodium in 75 ml. of methanol) was added 28 g. of ethyl acetoacetate and then 29 g. of phenylhydrazine was added and the mixture was refluxed for fifty hours. After cooling, 600

(4) Weissberger and Porter, *THIS JOURNAL*, **64**, 2133 (1942).

ml. of 1% sodium hydroxide was added, forming a thick precipitate. This suspension was poured into 1 liter of cold water containing 60 ml. of glacial acetic acid. After stirring for half an hour, the product was filtered and washed with water to yield 50 g. of tan powder; m. p. 130–150°.

The product was recrystallized (Norite) from 400 ml. of 66% ethanol. The first crop of 21 g. was recrystallized from 200 ml. of 66% ethanol to give 14.7 g. of white granular crystals; m. p. 168–169°. Numerous recrystallizations of the crops obtained from the mother liquors gave a total of 2 g. more of good material; yield, 16.7 g. (30%).

Anal. Calcd. for $C_{13}H_{13}N_3O$: N, 16.7. Found: N, 16.52.

2. A mixture of 2 g. of 1-phenyl-3-hydroxy-5-pyrazolone imide¹ and 5 ml. of aniline was refluxed until the evolution of ammonia slacked off (two and one-half hours). After cooling, the mixture was taken up in 15 ml. of benzene and extracted with 30 ml. of 2% sodium hydroxide. Acidification with 5% hydrochloric acid gave a dark gum. This was extracted with 30 ml. of 50% ethanol on the steam-bath, and the solution decanted from an oil which separated on cooling. Further cooling gave 0.8 g. of tan crystals, which on recrystallization (Norite) from 15 ml. of 50% ethanol yielded 0.55 g. (20%) of white granular crystals; m. p. and mixed m. p. with the above (1) 167–168°. The 1-phenyl-3-hydroxy-5-pyrazolone anil is soluble in 3% sodium carbonate but insoluble in 2% hydrochloric acid.

1-Methyl-3-anilino-5-pyrazolone, VII.—To a solution of sodium methylate (1.43 g. of sodium in 15 ml. of methanol) was added 8.1 g. of ethyl acetoacetate, 8.4 g. of phenyl isothiocyanate, and the solution refluxed for half an hour.

To 9 g. of methylhydrazine sulfate suspended in 50 ml. of ethanol and 10 ml. of water was added 8.9 ml. of 40% sodium hydroxide, portionwise with vigorous shaking. The resulting sludge was added to the above solution and the mixture refluxed for four hours. It was then cooled and filtered (the residue was completely soluble in water) and the filtrate concentrated to 25 ml. The product crystallized on cooling, was filtered and recrystallized from 25 ml. of absolute ethanol to yield 1.3 g. (11.5%) of white needles; m. p. 220–222°. Another recrystallization did not raise the melting point.

Anal. Calcd. for $C_{10}H_{11}N_3O$: C, 63.5; H, 5.82; N, 22.2. Found: C, 63.32; H, 5.88; N, 22.07.

The 1-methyl-3-anilino-5-pyrazolone is soluble in 3% sodium carbonate and sparingly soluble in 2% hydrochloric acid.

1-Methyl-3-hydroxy-5-pyrazolone Anil, VIII.—The filtrate from the crude product in the preceding preparation was made acid to litmus with about 1 ml. of glacial acetic acid and poured into 100 ml. of cold water. The precipitated oily solid was washed with water, slurried with 10 ml. of benzene, and filtered. The crude product (2.1 g., m. p. 175–190°) was extracted by stirring on the steam-bath successively with 30 ml. and 20 ml., and finally by boiling with 10 ml. of water and filtering hot. The residue (0.4 g., m. p. 205–208°), recrystallized from 95% ethanol, gave 0.2 g. (2%) of white granular crystals; m. p. 208–209, mixed m. p. in equal amounts with the isomer obtained above, about 180°.

Anal. Calcd. for $C_{10}H_{11}N_3O$: C, 63.5; H, 5.82; N, 22.2. Found: C, 63.26; H, 5.61; N, 21.95.

The 1-methyl-3-hydroxy-5-pyrazolone anil is soluble in 3% sodium carbonate and in 2% hydrochloric acid.

Coupling Tests with *p*-Nitrosodimethylaniline.—One-tenth millimole each of coupler and *p*-nitrosodimethylaniline were refluxed in absolute ethanol (10–15 ml.) for two hours. If a magenta dye is formed, the solutions turn from green to a deep red within thirty minutes. Only a slight darkening of the original green color is noted when no dye is formed.

Summary

The procedure of Worrall for the formation of pyrazolone derivatives was modified. With the changed procedure were obtained the new compounds 1-phenyl-3-hydroxy-5-pyrazolone anil, 1-methyl-3-anilino-5-pyrazolone, and 1-methyl-3-hydroxy-5-pyrazolone anil.

The formation of azomethine dyes by condensation of *p*-nitrosodimethylaniline with pyrazolone derivatives as a test suitable for the distinction of isomeric compounds is described.

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